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FERTILIZATION IN *ULMUS* WITH SPECIAL REFERENCE TO HYBRIDIZATION PROCEDURE[1]

BY L. P. V. JOHNSON[2]

Abstract

Germination tests on fruits derived from open, self, and cross pollinations made in *Ulmus* during four seasons strongly indicate a high degree of self sterility, possibly due to protogyny. It is suggested that a hybridization procedure involving the cross pollination of non-emasculated flowers is feasible in many species of the genus, especially under conditions that permit large-scale crossing and the growing of fairly extensive populations from which selfed seedlings may be eliminated.

Introduction

The flowers of *Ulmus*, the elm, are small, perfect, and occur in close clusters. These characteristics proved a serious handicap in hybridization work based on the emasculation procedure. Since *Ulmus* is reported to be normally cross fertilized and to exhibit marked protogyny (1, 2) there appeared to be some prospects of finding a degree of self sterility* sufficient to obviate the necessity of emasculation. This led to the experiments reported herein.

Materials and Methods

The study was made upon a number of elms growing in roughly the same site at the Dominion Arboretum, Ottawa. The species involved were as follows: *Ulmus americana* L., *U. fulva* Michx., × *U. hollandica* Mill. (one specimen apparently var. *major* (Sm.) Rehd., another apparently var. *superba* (Morr.) Rehd.), *U. laevis* Pall., *U. procera* Salisb., and *U. pumila* L.

Three individuals of *U. americana* and two of *U. pumila* were used as maternal parents. Only single specimens of the other species and varieties occur in the Arboretum.

Several different kinds of covering were used in bagging, the most common being a glassine paper bag protected by a kraft paper bag. Cross pollination was effected by placing a few dehiscing flower clusters from a tree of the appropriate species within the bag. Seeds were germinated on moist blotters at 22° C.

[1] *Manuscript received August 9, 1945.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry, N.R.C. No. 1374.

[2] *Geneticist.*

* *"Self sterility" is used in the present paper to denote the practical condition of failure to self fertilize, whether due to self incompatability or to protogyny (female part of flower maturing before male part).*

Results

The experimental results are presented in Table I. The relative percentages of viable seeds obtained from the different types of fertilization strongly indicate a high degree of self sterility in each of the *Ulmus* species studied.

A measure of the effect of bagging on seed set and seed viability is provided by the data from cross pollinations. It is believed that the flowers bagged for selfing developed under conditions comparable in every way to those provided for flowers bagged for crossing—except that self pollen only was available.

It is important to note that the viability of seeds derived from open pollinations varies greatly from year to year. This is believed to be related to seasonal differences in the conditions that promote cross fertilization (rain-free periods, winds, etc.). An observation supporting this view was made during the flowering period in 1945—a period of almost continuous rain and high humidity. Two trees, × *U. hollandica* var. *superba* and *U. pumila*, grow side by side in the Arboretum. The side of × *U. hollandica* next to, and with some branches touching, *U. pumila* was heavily laden with viable seeds, while the other side produced only a light yield of poor seed. Presumably, under weather conditions that permitted little pollen dissemination, the tree, being largely self sterile, was able to set viable seed only on the side with a close-at-hand source of suitable foreign pollen.

TABLE I

GERMINATION RESULTS FOR SEEDS DERIVED FROM OPEN, SELF, AND CROSS POLLINATIONS IN *Ulmus* SPECIES

Material	Total No. of:		Viable seeds, %				
	Bags	Fruits*	1939	1940	1941	1944	Average
U. americana, open pollinated	—	487	30.8	36.0	46.4	4.1	27.7
selfed	53	1582	0.2	3.6	3.5	0.0	1.5
× *U. americana*	29	947	56.7	33.3	23.0	2.6	25.6
U. fulva, open pollinated	—	200	1.6	19.6	—	—	10.6
selfed	10	179	0.0	0.0	—	—	0.0
× *U. americana*	10	261	7.7	6.2	—	—	7.1
U. laevis, open pollinated	—	348	24.0	70.0	35.4	3.0	31.7
selfed	18	540	0.0	0.0	1.3	0.5	0.4
× *U. americana*	13	827	58.4	42.0	27.8	1.2	35.9
U. procera, open pollinated	—	70	—	—	—	6.0	6.0
selfed	8	40	—	—	—	0.0	0.0
× *U. pumila*	6	52	—	—	—	13.4	13.4
U. pumila, open pollinated	—	400	8.0	22.0	5.0	53.0	22.0
selfed	38	1268	0.0	0.0	0.7	2.3	0.9
× (×) *U. hollandica major*	33	1159	6.1	22.8	4.8	12.1	10.9

* *Includes all fruits collected whether empty or fully developed.*

Discussion and Conclusions

The objective of the work, to find if self sterility existed in *Ulmus* to a degree sufficient to obviate the necessity for emasculation in the hybridization technique, has been attained. It is concluded that such a degree of self sterility does exist. The fact that elm seedlings of undoubted hybridity have been produced by bagging and cross pollinating non-emasculated flowers speaks for itself.

Whether this self sterility represents true self incompatibility or is merely due to protogyny is left an open question.

It has been observed, however, that the proportion of selfed seedlings in populations derived from interspecific cross pollinations is higher than would be expected on the basis of the results in Table I. This is attributed to some stimulatory effect on the stigma, produced by foreign pollen not entirely compatible with the maternal species, resulting in increased receptivity to self pollen.

The practical conclusion is that a hybridization procedure involving non-emasculated flowers may be recommended for *Ulmus*, under conditions that permit large-scale crossing and the growing of fairly extensive populations from which the selfed seedlings may be eliminated.

Acknowledgments

The author acknowledges the assistance of Mr. E. C. Bradley in the field work of the investigation, of Dr. H. A. Senn in identifying the material, and of Mr. M. W. Thistle and Dr. Margaret L. Landes in the preparation of the manuscript.

References

1. East, E. M. Proc. Am. Phil. Soc. 82 : 449-518. 1940.
2. Knuth, P. Handbook of flower pollination, Vol. III (Translation by J. R. Ainsworth Davis). Clarendon Press, Oxford. 1908.

TETRAPLOIDY IN FLAX[1]

By J. G. Ross[2] and J. W. Boyes[3]

Abstract

Tetraploid forms of the flax variety, Redwing, and the F_1 flax hybrid, Bison × Redwing, were most easily obtained by treatment of the epicotyl of seedlings with colchicine in lanolin emulsion. Seeds of the tetraploid forms were considerably larger than those of the diploid; but, because of their lower fertility and later maturity, yield and quality were definitely inferior.

Introduction

Doubling the chromosome number of plants by means of colchicine treatment was investigated as a means of obtaining a large-seeded oil flax adapted to conditions in western Canada. Increased seed size of the tetraploid over the diploid forms has been reported in flax (3) and in numerous other plant species (1, 2). Fertility, however, may be decreased in autotetraploid forms. Though this possibility was recognized, the ease with which tetraploid plants can be obtained prompted the present investigation.

Results

Plants of the variety Redwing and the F_1 hybrid of the cross Bison × Redwing were obtained in the tetraploid form after colchicine treatment of large numbers of seeds and seedlings. The application of colchicine in lanolin emulsion to the epicotyl of very young seedlings gave a larger proportion of tetraploid plants than did any other method. Increase in the size of pollen and stomata, characteristic of tetraploid plants, as shown in Table I, was used as a means of identifying the tetraploids. The identity of these plants was confirmed by chromosome counts: diploid plants had 30 chromosomes, while the tetraploid plants had approximately 60. The majority of these tetraploid plants had the exact $4n$ number of 60 chromosomes.

Comparisons of the tetraploids with the diploids were made in 1942, 1943, and 1944 and are shown in Tables I, II, and III. In 1944, though their number of days to blooming was not consistently greater, the tetraploids matured much later than did the diploids. In that year, results also indicated that the tetraploids had a tendency toward weaker straw and were slightly shorter than the diploid forms. Flower size and seed size were considerably larger in the tetraploids, but seed set was greatly reduced. This is shown in Tables I and II by the low average number of tetraploid seeds per boll, and in Table III by the low yields of the tetraploids. In the quality tests conducted in 1943 and 1944 the tetraploids, in comparison with the diploids,

[1] *Manuscript received September 17, 1945.*

Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alta., with financial assistance from a University of Alberta Research Scholarship.

[2] *Graduate Assistant in Cereal Breeding, University of Alberta.*

[3] *Formerly Assistant Professor of Field Crops, University of Alberta; now Assistant Professor of Genetics, McGill University, Montreal, Que.*

were definitely inferior in oil content, equal or slightly inferior in drying quality, and approximately equal in percentage nitrogen. Though seed size was increased by doubling the chromosome number, as is shown by the larger

TABLE I

COMPARISON OF TETRAPLOID AND DIPLOID FLAX PLANTS OF THE VARIETY REDWING, GROWN IN THE GREENHOUSE IN 1942

—	No. of plants	No. of days to first flower	Flower diameter, mm.	Pollen diameter, mμ	Stomatal length, mμ	Height at maturity, in.	No. of seeds per boll	1000-kernel weight, gm.
Tetraploids	14	163	20	69	33	26.4	2.0	5.64
Diploids	13	144	14	55	25	27.4	7.1	4.78

TABLE II

COMPARISON OF SEED SET OF DIPLOID AND TETRAPLOID SHOOTS OF THE SAME PLANTS GROWN IN THE GREENHOUSE IN 1942 AND IN THE FIELD IN 1943

Variety	Diploid		Tetraploid	
	No. of bolls	Average no. of seeds per boll	No. of bolls	Average no. of seeds per boll
1942				
Redwing	6	7.5	4	2.1
Bison × Redwing	4	7.5	21	3.0
1943				
Redwing	200	8.0	200	3.0
Bison × Redwing	—	—	200	2.8

TABLE III

COMPARISON OF PLANT AND SEED CHARACTERISTICS OF TETRAPLOID AND DIPLOID FORMS OF FLAX IN THE YEARS 1943 AND 1944

Variety	Days to full bloom	Straw strength, (1-10)	Height, in.	Days to maturity	Yield, bu. per acre	Oil content, % dry basis	Iodine value, Wij's	1000-kernel weight, gm.	Nitrogen, % dry basis
1943									
Redwing 4n	—	—	—	—	—	35.7	190.5	6.77	4.29
Redwing 2n	—	—	—	—	—	39.5	196.8	5.07	4.19
1944									
Redwing 4n	56	8.0	24.0	127	10.4	36.9	192.7	—	4.24
Redwing 2n	53	10.0	25.2	99	30.0	40.5	193.6	—	4.25
Redwing × Bison 4n	54	9.0	23.0	121	10.4	37.3	193.9	—	4.43
Redwing × Bison 2n	57	8.5	25.2	109	25.2	40.2	193.1	—	4.26

size of tetraploid seed (Fig. 1) and by the increased 1000-kernel weight (Tables I and III), yet late and uneven maturity of the tetraploids led to the formation of many immature green seeds. Consequently the oil content was severely reduced.

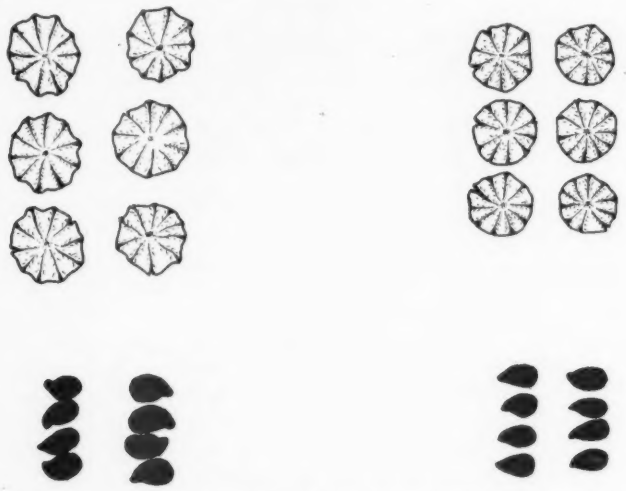

FIG. 1. *Illustration of the difference in size of flax bolls and seeds from a tetraploid plant on the left and from a diploid plant on the right. (1¼ × natural size).*

These results show that, even though seed size of flax may be increased by doubling the chromosome number, the consequent low fertility and late maturity of tetraploids prevent the immediate development of a more suitable variety by this method. It is possible, however, that fertility may be increased by selection.

References

1. BLAKESLEE, A. F. Effect of induced polyploidy in plants. Am. Naturalist, 75 : 117-135. 1941.
2. DERMEN, H. Colchicine polyploidy and technique. Botan. Rev. 6 : 599-635. 1940.
3. LUTKOV, A. N. Mass production of tetraploid flax plants by colchicine treatment. Compt. rend. acad. sci. U.R.S.S. 22 : 175-179. 1939.

GERMINATION OF SEEDS OF THREE SPECIES OF *AGROSTIS*[1]

By C. W. Leggatt[2]

Abstract

Germination tests on seed of the 1939 crop of *Agrostis stolonifera* L. var. *compacta* Hartm., *A tenuis* Sibth., and *A. canina* L. were begun in September of the same year and carried through to May, 1940, with the object of studying the drift, with time, of germination capacity and germination speed under different conditions.

Seeds of the three species proved to be highly light-sensitive but low light-requiring. Germination capacity was proportional to light intensity while germination speed was inversely proportional to light intensity. The possibility that excess of light (>200 ft-c. for seven and one-half hours daily) might depress germination capacity was suggested. There was no significant difference, in their effect on germination, between Mazda and Fluorescent "Daylite" illumination at equal foot candle intensity.

Potassium nitrate in 0.2% solution proved more effective than light in promoting germination in not fully germinating-ripe seeds but its use did not stimulate germination beyond the natural limits of fully ripe seed; thus its use in seed control laboratories is justified for these species. Its effect on the seed is clearly distinct, physiologically, from that of light.

An interesting phenomenon was observed, consisting in a falling off in germination capacity accompanied by an absolute increase in germination speed during the period February–March.

Seeds of these species fall into four classes. The interrelationships between these classes and changes within them and their connection with the February–March phenomenon are discussed in the text.

Introduction

The seeds of *Agrostis* have for some years been generally recognized as light-sensitive. Toole and Goss (8) in 1925 reported that the use of a 0.2% solution of potassium nitrate to moisten the substratum and exposure of the test to light during part of the day hastened the germination of some samples of *Agrostis* but that a sharp alternation of temperature daily was also necessary. In 1929 Thygeson (7) found that potassium nitrate solution gave results closely comparable to results obtained on soil in uncovered Petri dishes, the seeds being barely covered with soil, and concluded that the use of nitrate was justifiable as a germination medium for seeds of *Agrostis*.

Gadd (3) in 1939 pointed out that *Agrostis* seed in most years is not germinating-ripe on which account his laboratory puts in a double test on all samples, using an 18° to 36° C. temperature alternation for one and 10° to 36° C. for the other. Samples not germinating-ripe under standard conditions germinate very satisfactorily under this latter alternation.

[1] *Manuscript received September 14, 1945.*

Contribution from the Seed Research Laboratory, Division of Plant Products, Department of Agriculture, Ottawa.

[2] *In charge of Seed Research.*

Note:—This paper was practically ready for publication in 1942, when the author received leave of absence to engage in special war duties. An abstract of the paper was presented in the A.O.S.A. News Letter for December, 1942. In order no longer to delay publication, a review of more recent literature, than was then available, has not been made.

In the Canadian laboratories similar difficulties have been found and have frequently been the cause of failure of different tests on the same material to corroborate one another. Elliott (1) reported receiving in August, 1932, a number of samples of red top (*A. stolonifera* L. var. *major* Gaud. Far.) that were not germinating-ripe. The average germination of five samples was 74%. By November of the same year the germination had risen to 90%. In 1937 he reported receiving a sample of brown top (*A. tenuis* Sibth.), which germinated 47% on the original test, a retest giving 63% while a test using 0.2% nitrate gave 90%. On the same sample he reports the Sackville laboratory as having obtained 96% using the aluminium cup method and 88% by the standard method, on top of blotters at 20° to 30° C. in light in the Jacobsen apparatus.

As a result of the higher tests that were being obtained at Sackville than in several other laboratories in the United States and Canada, Hope (4) in 1936 conducted an investigation on the effect of full daylight compared with the subdued light that was used in some laboratories. He used samples of creeping bent grass (*A. stolonifera* L. var. *compacta* Hartm.) of New Brunswick origin and of the 1935 crop, starting the tests in June, 1936. The samples should have been fully germinating-ripe at that date but it was found necessary to prolong the tests beyond the customary time. He used both the top of blotter and the aluminium cup method under both sets of light conditions, the full daylight being given by placing the germinators close to a bright window while the subdued light was the result of placing them at the back of the room. He found that in full light the average of five samples gave 84% on top of blotters and 90% by the cup method in 35 days; in subdued light the figures were 34% and 38%, respectively after 49 days.

In a comparison of the top of blotter and cup methods he reported a mean difference with 15 samples of *Agrostis* (species not mentioned) of 10% in favour of the latter (5) and made the observation that the seedlings also appeared stronger. In 1938 (6) he reported comparisons of tests run at 30° C. constant with the standard 20° to 30° C. alternation in which the high temperature tests showed a marked inhibition.

It is clear from the foregoing that the seeds of *Agrostis* species found in commerce are highly sensitive to germinating conditions and that they retain this sensitivity long after the normal after-ripening period has been completed. Some of the results suggest that differences in light intensity have a marked effect on the germination figure such that laboratories in districts that have prolonged periods of dull weather may be unable to obtain complete germination at all times of the year. Just what are the limits in light and temperature sensitiveness have not been very clearly brought out. The investigation to be reported in this paper has been carried out with the intention of gaining further information on these points.

Materials and Methods

Through the courtesy of Mr. A. Hope of the Sackville laboratory, a sample of creeping bent grass collected in August, 1939, on the Tantramar marshes near Sackville, N.B., and one of brown top also collected in the same month at Montecello, P.E.I., were received on the ninth day of September, 1939, for the purpose of this investigation. On the 23rd of October, 1939, a sample of velvet bent grass (*A. canina* L.) mixed with brown top grown the same year on Prince Edward Island was also received from him. The investigation was put in hand forthwith upon receipt of the material.

The material was first cleaned on the analytical blower and only such 'seeds' as contained caryopses were used.

Under each set of conditions 2 × 100 seeds were used.

Three media were used; sand, Kimpak, and filter paper (Whatman No. 3), the seeds being planted on top of the medium in each case. While the sand generally gave somewhat better results, there was insufficient difference to justify separate treatment of the media in the analysis of the results. Thus the results reported are the averages of 6 × 100 seeds except in the case of the velvet bent of which the separation from the brown top was so time-consuming that it was only feasible to put in 2 × 100, using sand only as the medium.

All tests were carried out in 90 × 15 mm. covered Petri dishes.

Moisture was controlled by eye, just sufficient being added to give the surface of the medium a slightly shiny appearance, it having previously been found that by this means a closer control is possible than by measurement, on account of variations in the medium itself and in size and contour of the dishes. In the case of the filter paper tests, 9-cm. filter papers were placed on three thicknesses of germination blotter cut octagonally so as to leave little spaces between the sides of the octagon and the circumference of the dish. These spaces served as 'wells' to hold surplus water and helped to keep the filter papers at the most favourable moisture level.

The tests were held under conditions of controlled temperature and light. In all cases the low temperature experience lasted 16½ hr. daily and the high temperature seven and one-half hours. Light experience, where given, was during the high temperature period. The Mazda lamps used developed so much heat that a water heat-filter 2 to 3 in. in depth had to be employed; light intensities were measured at the seed level after passage through the filter. The tests in 'subdued light' were exposed for very brief periods daily to the light in a dark part of the room (not more than 5 ft-c.) when being transferred from one germinator to another, no particular precautions having been used to exclude light. During the high temperature experience they were exposed to light too feeble to be measured by the photronic cell used for the light measurements. The daylight fluorescent lamps employed developed so little heat that no filter was necessary. Optically, the Mazda light was quite yellow in comparison with the fluorescent.

'Dark' tests were placed in motion picture film tins as soon as planted; these were then placed in dark controlled-temperature cabinets.

It should be remarked that in the dark tests, light was completely excluded only up to the seventh day, when the first count was made. The dark tests were then opened behind a shade where there was only just enough light to see to make the count.

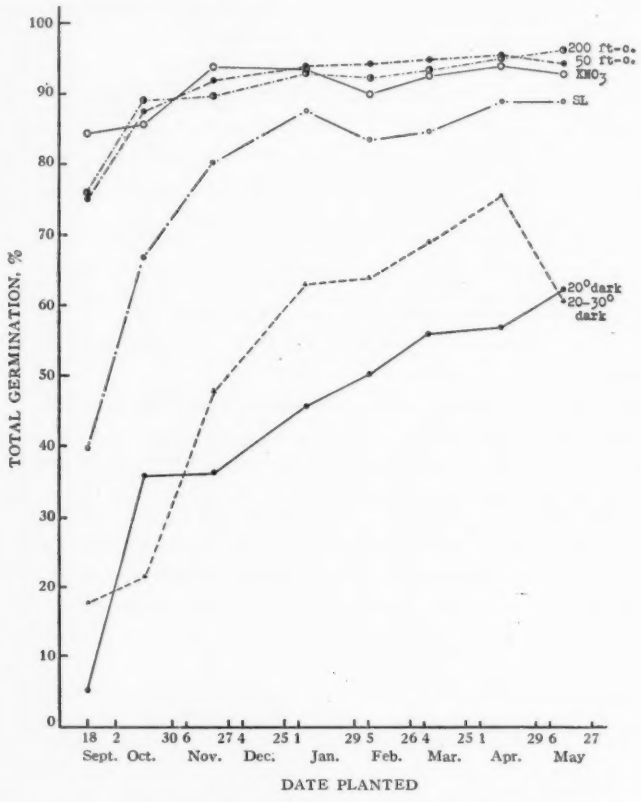

FIG. 1. *A stolonifera L. var. compacta Hartm. Total germination after 21 days of tests planted at, approximately, monthly intervals under various conditions.*

Legend for Figs. 1 to 6:

- *20° C. constant temperature, dark;*
- *20° to 30° C., dark;*
- *subdued light;*
- *subdued light with KNO_3;*
- *50 ft-c. for 7½ hr. daily;*
- *200 ft-c. for 7½ hr. daily.*

All tests except the first were made at a temperature alternation of 20° C. for 16½ hr. and 30° C. for 7½ hr. daily.

Where alternating temperatures were used, the tests were transferred from a germinator held at the one temperature to one held at the other.

The following sets of conditions were used:—

(*a*) 20° C. constant, dark.
(*b*) 20° to 30° C., dark.
(*c*) 20° to 30° C., subdued light.
(*d*) 20° to 30° C., 50 ft-c., fluorescent lamps.
(*e*) 20° to 30° C., 200 ft-c., Mazda lamps.
(*f*) 20° to 30° C., subdued light, 0.2% nitrate solution instead of distilled water.
(*g*) 20° to 30° C., 50 ft-c., Mazda lamps.

The whole series of tests was repeated at, approximately, monthly intervals. In order to distribute the work, the planting dates were staggered but the time drift was not sufficiently swift for the differences in dates to reveal appreciable differences in response. Germination counts were made at 4, 7, 10, 14, and 21 days.

Results

Part 1. Drift of Germination Response with Time; Final Counts

Creeping Bent Grass

In Fig. 1 are shown the final results, after 21 days, of samples set to germinate at various dates from 18th September, 1939, to 13th May, 1940.

The sample is of high germination capacity, a test of over 96% having been obtained at the last date of planting. It is evident, however, that it was not fully germinating-ripe by any of the methods used until the planting of 21st November when the test with nitrate in subdued light gave 94%, the highest germination obtained at that date. There is little difference between the nitrate, 50 ft-c., and 200 ft-c. tests from that date on. The tests in subdued light appear to reach their maxima by the 4th January which however is approximately 6% lower than those of the more favourable conditions, a difference that must be considered highly significant ($P < .01$).

The tests in the dark reveal a marked depressive effect, which is only in small measure compensated by a temperature alternation. It should be noted, however, that the average temperature in the alternation is about 3° C. higher than the constant temperature used in the dark tests.

Brown Top

Fig. 2 gives the data for this species. This sample is also of high germination capacity, a test of nearly 95% having been obtained at the last planting. At no stage, however, was it as germinating-unripe as the creeping bent and the tests in subdued light became effectively equivalent to the tests with light or nitrate by the middle of January.

In this species also complete exclusion of light exerts a markedly depressing effect, only partially, though to a greater degree than in creeping bent, compensated by alternation of temperature.

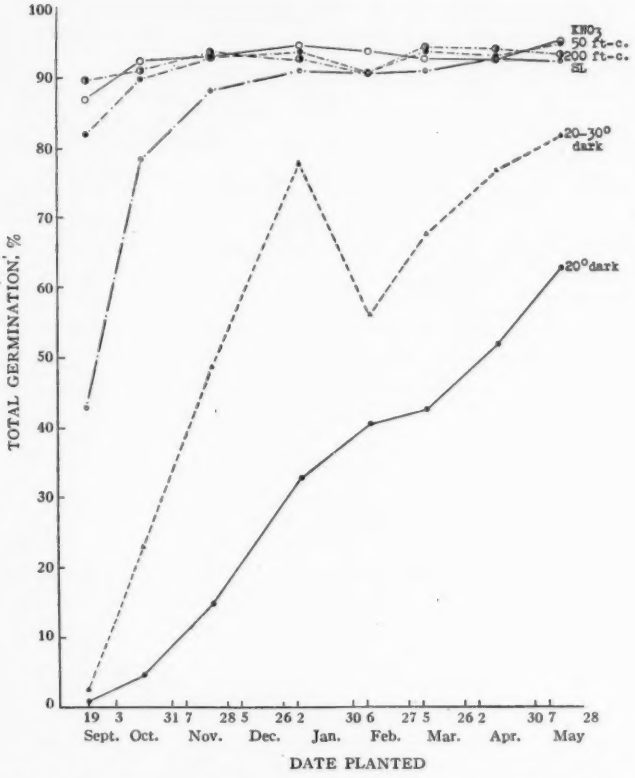

FIG. 2. *A. tenuis Sibth. Total germination after 21 days, of tests under various conditions planted at, approximately, monthly intervals. For legend see Fig. 1.*

Velvet Bent Grass

This sample was received at a later date than the other two, so that the first date of planting is towards the end of October. Fig. 3, in which the results for this species are presented, should therefore be regarded with this in mind. It was clearly at a somewhat later stage of after-ripening at the beginning of the investigation and the results of the first series of tests are comparable with those at the same date of the creeping bent grass. The behaviour in general is intermediate between that of the creeping bent grass and the brown top. Again the depressive effect of complete exclusion of light with partial compensation brought about by alternating temperature is in evidence.

Discussion, Part 1

A curious feature common to all these graphs, which is too consistent to be due to sampling error, is believed not to be due to the conditions of the

test since all controls were functioning perfectly, and which thus cannot be considered as accidental, is a relative lowering of the germination capacity during the period February–March. It appears that a partial secondary dormancy sets in at this period, a possible cause for which is discussed later

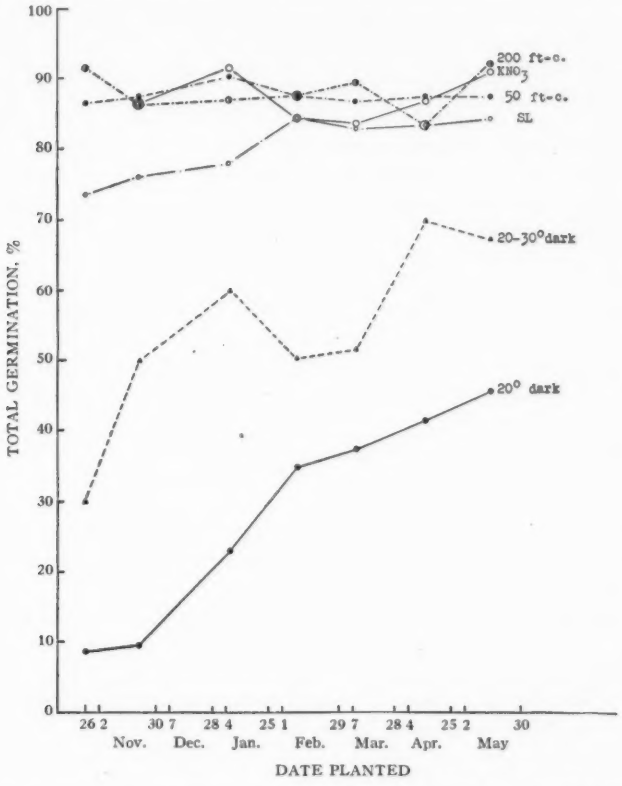

FIG. 3. *A. canina L. Total germination after 21 days, of tests under various conditions planted at, approximately, monthly intervals. For legend see Fig. 1.*

in this paper. Under the most favourable germinating conditions the magnitude of this effect is not great enough to be of serious concern to the seed analyst.

It will be seen from later discussions that 'after-ripening' is a relative term but if for the moment we consider that after-ripening has been completed when the tests given some degree of light have reached their maximum germination, then it may be said that the process lasts until about the turn of the year. Since the tests in subdued light nearly attain the figures given by those with adequate light and, in the case of Brown Top, actually do so,

the three samples may be said to be moderately *low-light–requiring*, while the fact that such a marked depression of germination capacity occurs with complete exclusion of light indicates that they are *highly light-sensitive*. It was unfortunately not possible, on this occasion, to obtain samples of different degrees of maturity; the picture might be different with less mature seed. However, it may be said that their maturity was such as would usually be found in seed going through the trade. It is possible, therefore, that intensity of light may not have had as important a role in the germination of *Agrostis* seeds as has been suggested, even though some light is essential. The conditions of 'subdued light' are of an intensity far lower than could possibly be found in any seed germination laboratory where the seed was given a test in light, which is the standard method for the genus. While the investigations discussed have not served to reveal the lower limit of light intensity at which the maximum germination capacity of after-ripened samples is reached it can be said that an adequate intensity may be provided by placing a 100-watt incandescent lamp, three feet above the level of the seeds placed in a Jacobsen apparatus. Seeds that are in the germination-unripe condition may require the extreme temperature fluctuation suggested by Gadd but time and germinator space did not permit extending the scope of this investigation.

The fact that Hope (4) obtained such a marked difference in germination between seeds placed near a bright window and those placed at the back of the room may have been due to a differential effect of light under the conditions of temperature control available to him. Where conditions are otherwise relatively less favourable, the effect of light may be to counterbalance other (unfavourable) influences, as has been found by the author for lettuce and bluegrass. In the present investigation a sharp alternation of temperature, accurately controlled, has been given.

The aluminium cup method used by Hope to which reference has been made earlier in this paper has the effect of providing a plentiful supply of moisture for the seed without preventing free access of air. The same result is accomplished by the sand tests as used in this investigation and this probably accounts for the somewhat better results obtained by this method than by the other two.

Part 2. Germination Speed

Because there were great differences between the absolute germination values by different methods and because the speed of germination for the purpose of this study is the proportion of the total germination that occurred early in the test, the actual germination figures were first transformed into the percentage, at each count, of the total germination by the method at the date in question.

For example, the transformation for creeping bent grass planted on the 16th October, 1939, using nitrate, is as follows:—

Days	4	7	10	14	21
Germination, %	12.5	72.0	79.5	80.5	82.5
Speed, %	15	87	96	98	100

As a comparative measure of germination speed the test giving the highest average germination speed was picked out and rated 100%. Each of the other tests was compared with it by finding the deviation from it at each count, summing, dividing by the number of counts and subtracting the result from 100. Since there were considerable differences between the media in the earlier counts of each test, only the sand tests, which in general were superior, have been used in the speed of germination comparisons.

The data so obtained are shown in Figs. 4, 5, and 6 for creeping bent grass, brown top, and velvet bent grass, respectively.

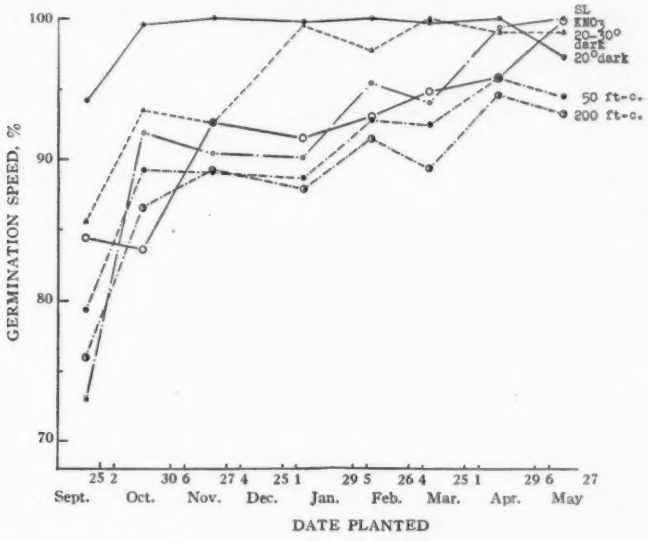

FIG. 4. *A. stolonifera L. var. compacta Hartm. Germination speed of tests planted at, approximately, monthly intervals under various conditions. For legend see Fig. 1.*

Discussion, Part 2

In general there is evident an increase of germination speed with time or with the progress of after-ripening, as would be expected. The curves are steep at first, gradually becoming less so, but not always reaching their possible maxima. It should be borne in mind that the data are relative and that it is therefore possible that even the method giving the highest speed and so rated at 100% might have given an even higher speed if the tests could have been continued during succeeding months.

The curves show another feature in common, with one or two exceptions, namely, a sharp rise in February followed by a subsequent depression before the rise occurs again. This should be noted in connection with the dip in the curves for total germination at the same date previously referred to, and will be discussed later.

The February rise is not shared by the nitrate tests except in the doubtful case of Velvet bent grass where the form of the curve appears to be a little exaggerated. Even in this case the rise is not very great nor the subsequent depression very pronounced.

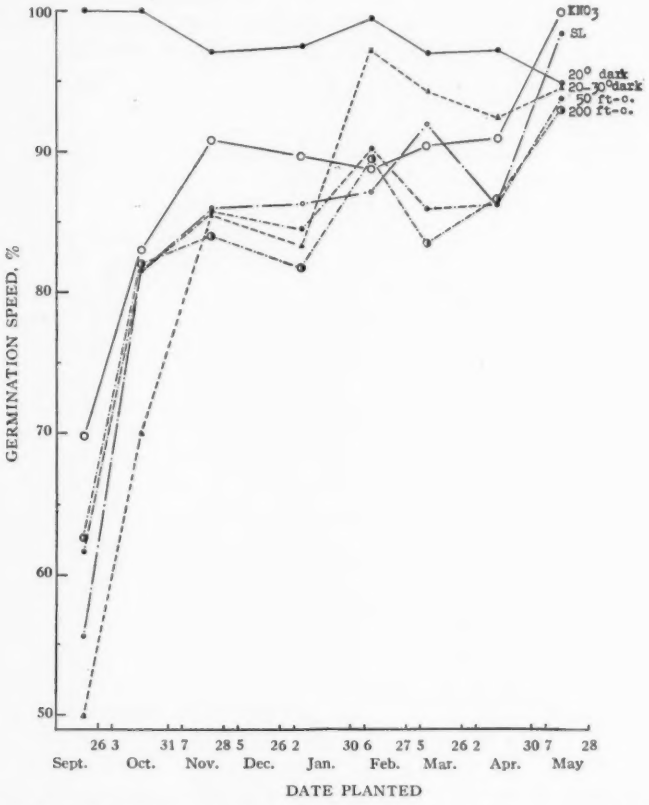

FIG. 5. *A. tenuis Sibth. Germination speed of tests planted at, approximately, monthly intervals under various conditions. For legend see Fig. 1.*

Significance of Data

The curves for 20° C. constant temperature in total absence of light do not share in the tendency towards an upward drift with time. They maintain a fairly consistent and high level, close to 100%. That is, all the germination that is going to occur does so early in the test. (It should be mentioned here that the four-day count was not made on the completely dark tests, in order that the whole of the (presumed) most light-sensitive period of the test might be carried through in complete darkness. Thus the earliest count given was

at seven days. These curves are therefore not strictly comparable to those of tests in which any light experience was given.) The conclusion may be drawn from this that there is a certain proportion of seeds at any date that are germinating-ripe at low temperature in the absence of light, that they

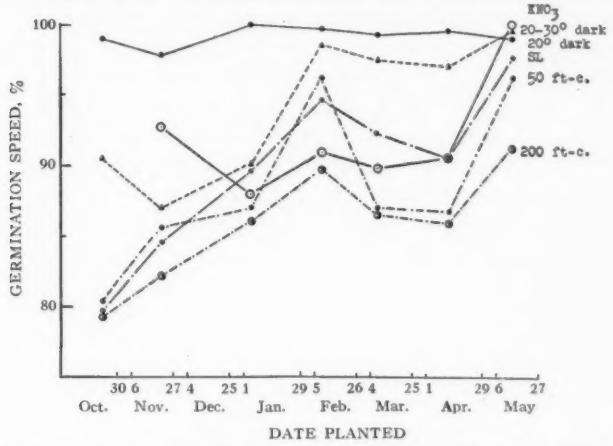

FIG. 6. *A. canina L. Germination speed of tests planted at, approximately, monthly intervals under various conditions. For legend see Fig. 1.*

germinate promptly, and that the remainder remain completely dormant. The cleavage between the two kinds of seeds is surprisingly sharp and rather striking. That the exposure to light that occurs during the counts made at 7, 10, and 14 days is not responsible for this, on account of a possible inhibiting effect of light at the constant low temperature, was proved by duplicate tests given only one count, at 21 days, which did not differ significantly from those given the intermediate counts.

The proportion of such germinating-ripe seeds increases, of course, with time (cf. Figs. 1, 2, and 3). For purposes of reference these will be referred to as Class *A*.

The curves of germination speed for 20° to 30° C. temperature alternation in complete absence of light do share in the general upward drift with time. The corresponding curves for total germination, however, are at a somewhat higher level generally than those of the 20° C. constant temperature. The difference may be interpreted as a proportion of seeds for which light is not necessary but that may be brought to germinate by a temperature alternation. This proportion will be called Class *B*. The higher initial germination at 20° to 30° C. indicates that some Class *B* seeds are brought to germinate quickly but the lower germination speed values show that it requires time to bring some of them to germination.

In order to attempt to present a picture of what happens during the process of after-ripening, the species may be considered separately.

Creeping Bent Grass (Fig. 4)

In this case the initial germination speed at 20° to 30° C. in the dark is fairly high, nearly 86%. It increased rapidly until by January it was practically equal to that at 20° constant and remained so until the end. There is a difference of 17% in total germination, however, in favour of the alternation (Fig. 1), a difference that is substantially maintained except for the reading in May when breakdown of temperature control rendered some of the results uncertain. There is therefore, from January on, a proportion of seeds, 15 to 17%, which are Class *B* but which are as prompt as Class *A* to germinate. There is here, then, an after-ripening change in Class *B* seeds as the season progresses.

Considering now the tests at a temperature alternation in light, it is seen that the germination speed curves run closely parallel and that they are spaced *inversely as the intensity of light.* Reference to Fig. 1 shows that the total germination on the whole is higher with greater light intensity, except that in five readings out of eight, 50 ft-c. is more favourable than 200 ft-c. These observations as to speed and total germination indicate that light tends to depress germination speed and that the higher intensity of light may tend to inhibit, slightly, the germination process.

As Classes *A* and *B* have been designated for convenience in reference, so we may designate a Class *C*, which is brought to germination under the influence of light and temperature alternation and a Class *D*, which does not germinate even under the influence of light. Since 100% germination was never attained with this material, an unknown number of Class *D* may be dead. Class *D*, of fully dormant seeds, is the ultimate source of supply for Classes *A*, *B*, and *C*.

The 'February dip' in the total germination curves in conjunction with the corresponding rise in the speed curves appears to be of some significance. The dip is barely in evidence in the curves for 20° dark and for 50 ft-c. and 200 ft-c., while the dark and subdued light tests at alternating temperature, including nitrate, show the dip clearly. This suggests that it is not Class *A* that is affected nor Class *C* but Class *B*. The higher relative germination speed is not simply the reflection of a lower total germination; a significantly greater number of sprouts grew by the first count than in either the preceding or succeeding tests and the rise in speed is shown by tests that gave little or no dip. This suggests that at this stage of the after-ripening process the normal course would have been that of increased speed of germination and of increased total germination where possible, but that in absence of sufficient light a partial secondary dormancy supervened after the germination process had started—possibly owing to accumulation of respiratory products formed in greater quantity than normal as a result of the greater germination speed coupled with lack of full development of the mechanism for neutralizing the effect of these respiratory products among a proportion of seeds just having passed into Class *B*.

Influence of Nitrate

That Class *B* seeds are not influenced by nitrate is suggested by the fact that the nitrate tests share in the February dip as do the other tests at 20° to 30° C., in subdued light or complete darkness, and Class *B* seeds appear to be responsible for the dip; that Class *D* seeds are so influenced is shown by the fact that higher germination is obtained with the nitrate early in the series than by tests in light; that Class *C* seeds are influenced appears from the fact that the subdued light tests and tests identical except for the use of nitrate run closely parallel after December (Fig. 1) though separated by about 5% in favour of the nitrate.

These observations are not true for *A. tenuis*, which suggests that a number of borderline *B* class seeds exist at this time in this species. This is borne out by the sudden and great increase in Class *B*. Such borderline seeds might still be affected by nitrate, thus lowering the effective number of seeds "Class B with respect to nitrate." In this case a nitrate 'dip' would not necessarily be expected. Note also that *A. tenuis* is *lower light-requiring* than the other species so that the subdued light to which the nitrate tests were exposed is relatively more effective.

The fact that the nitrate tests do not show an increase of germination speed but may even show a somewhat lower speed than would otherwise be normal for nitrate tests at this time suggests that a secondary dormancy is induced among a proportion of Class *C* seeds, which may be overcome by light, possibly as a result of an increase of respiration rate brought about by the nitrate.

Nitrate, while serving in the practical germination test as a partial substitute for light, appears to have an entirely different physiological effect.

Brown Top (Fig. 5) *and Velvet Bent Grass* (Fig. 6)

The picture is not significantly different for these two species except as mentioned above for brown top, though the argument has been developed from the results for creeping bent grass alone because they seemed a little more clear-cut. Fig. 5 compares fairly closely with Fig. 4, the chief differences being that the curve for 20° to 30° C. in the dark starts at a much lower point in the case of brown top and lags considerably in comparison with that for creeping bent until February, when it shows a peak in contrast to a dip. At the first date the relative positions of the curves of the tests given some light are the reverse of what they are at the last. The 20° C. constant curve shows a marked peak in February. In this curve also there is a tendency towards a slight fall in germination speed with time, the significance of which is questionable. The curves of Fig. 5 also show a rather stronger tendency towards a pre-February depression while the peak for the curve of 20° to 30° C. in subdued light occurs in March rather than February. In the case of velvet bent grass, Fig. 6, there are no results for September nor in October for the

nitrate tests, so that the earliest stages of after-ripening cannot be compared. However, the curves when examined in detail do not present a greatly different picture from those of the other two species, the chief differences being a somewhat more strongly marked post-February depression of germination speed and, for the test at 50 ft-c., a relatively greater February peak. All these differences can be ascribed to differences of degree or date rather than of kind and the general picture remains unaltered.

The data are too voluminous to present in detail, but specimen data on final counts on *A. tenuis* are presented in Table I.

TABLE I

GERMINATION OF *Agrostis tenuis* SIBTH. FROM SEPTEMBER, 1939, TO MAY, 1940. TOTAL PERCENTAGE GERMINATION IN 21 DAYS, BY MONTHS

Medium	Temp.	Light	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Sand			2.0	4.5	17.5	—	35.5	39.5	44.0	48.0	61.0
Kimpak	20° C.	None	0.0	6.5	13.0	—	26.5	37.5	42.0	45.0	59.1
Filter paper			0.5	2.0	14.0	—	35.5	44.0	41.5	61.5	67.5
Sand	20° to		2.0	22.5	54.5	—	72.0**	58.0	66.0	80.0	80.0
Kimpak	30° C.	None	3.5	23.0	41.0	—	74.0**	55.0	67.0	70.0	83.5
Filter paper			2.5	23.5	51.0	—	87.0**	55.0	69.5	79.6	80.5
Sand	20° to		50.0	76.5	90.8	—	92.5	88.0	94.0	91.0	91.0
Kimpak	30° C.	*SL**	34.5	79.5	83.0	—	90.0	93.0	89.0	91.5	91.0
Filter paper			45.0	79.0	90.5	—	90.0	93.5	89.5	95.5	93.5
Sand	20° to		85.5	91.0	93.5	—	96.0	96.0	89.5	—	—
Kimpak	30° C.	50 ft-c.	81.5	92.5	93.5	—	90.0	91.0	94.5	—	—
Filter paper			79.0	88.0	90.0	—	94.0	94.0	96.5	—	—
Sand	20° to	200 ft-c.	90.0	90.5	90.5	—	92.0	90.5	90.5	95.5	92.5
Kimpak	30° C.		87.5	91.5	95.0	—	92.0	91.0	95.5	92.0	92.5
Filter paper			91.0	91.0	94.5	—	94.0	91.0	96.0	93.5	94.0
Sand	20° to	*SL*	86.0	93.5	93.5	—	94.5	91.0	95.0	91.0	94.0
Kimpak	30° C.	and	—	91.0	94.5	—	95.0	93.5	90.5	93.0	95.0
Filter paper		KNO_3	88.0	92.5	90.5	—	94.0	96.5	92.0	93.0	95.0
Sand	20° to	50 ft-c.	—	92.0	93.0	—	94.5	88.0	94.5	95.5	95.5
Kimpak	30° C.	fluorescent	—	93.0	91.0	—	93.5	92.5	93.0	89.5	95.5
Filter paper		light	—	84.5	94.0	—	92.5	91.0	93.5	93.5	92.5

* *SL = Subdued light.*

** *Tests left at 30° C. during night of 10/1/40 in error.*

Note: All tests were affected in May on the 7th, 8th, and 15th days after planting by breakdown of temperature control. Temperatures in the morning were found to be about 30° C.

Acknowledgment

My thanks are due to my assistant, Miss A. W. Anderson, for her painstaking care in planting and counting the tests and to Dr. H. T. Güssow, formerly Dominion Botantist, and Dr. H. B. Sifton, Department of Botany, University of Toronto, for a critical reading of the manuscript.

References

1. ELLIOTT, G. A. Can. Seed Lab. News Letter, Nov. 1932.
2. ELLIOTT, G. A. Can. Seed Lab. News Letter, Feb. 1937.
3. GADD, I. Proc. Intern. Seed Testing Assoc. 11 (2) : 96-107. 1939.
4. HOPE, A. Can. Seed Lab. News Letter, July–Aug. 1936.
5. HOPE, A. Can. Seed Lab. News Letter, Jan. 1937.
6. HOPE, A. Can. Seed Lab. News Letter, June. 1938.
7. THYGESON, C. C. Proc. Assoc. Official Seed Analysts. 22nd Meeting, 43-46. 1929.
8. TOOLE, E. H. and GOSS, W. L. Proc. Assoc. Official Seed Analysts. 17th Meeting, 31. 1925.

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